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LARVAL CESTODES FROM POLYNEMID FISHES OFF VISAKHAPATNAM COAST, BAY OF BENGAL, ANDHRA PRADESH, INDIA

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ABSTRACT : Marine fishes serves as an intermediate hosts for many tetraphyllid and trypanorhynchid cestodes whose adults are mostly observed in elasmobranchs. However, a complete information of the life-cycle patterns of the tetraphyllid and trypanorhynchid cestodes are still lacking. Various developmental stages of larval cestodes such as *Scolex pleuronectis* and plerocercoids of trypanorhynchids analysed from a total sample of 1456 fish specimens belonging to four genera of the family Polynemidae of Visakhapatnam coast, Bay of Bengal during July, 2005 to June, 2007. Seven different types of *Scolex pleuronectis* (n= 3870) of the order Tetraphyllidae i.e., *Scolex pleuronectis* I-V stages, *Scolex pleuronectis bilocularis* Wagener, 1854, *Scolex pleuronectis trilocularis* Wagener, 1854, plerocercoid of trypanorhynchid spp. (n = 15) and larva of *Nybelinia* sp. (n= 17) were obtained from the stomach and intestinal walls of the host fishes. Cestode larvae in a fish prove their role as an intermediate host for a definitive host in which these larvae metamorphose to adult stages. Since adult tetraphyllideans and trypanorhynchids parasitize only elasmobranch fish, the occurrence of these larvae suggests that these fish are preyed by elasmobranch fish. This type of life-cycle studies are timely required to know the complete life-cycle patterns of these tetraphyllid and trypanorhynchid cestodes.

Key words : *Scolex pleuronectis*, Plerocercoid of *Trypanorhynchid* sp., larva of *Nybelinia* sp., Visakhapatnam coast.

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INTRODUCTION

The Bay of Bengal is the one of the main hub of global marine biodiversity supporting nearly 529 marine and 149 estuarine fish species and possesses high potential for further stock improvement (Islam, 2003; Amin and Kawsar, 2003). But the parasite fauna of these marine fishes in some coastal areas of Bay of Bengal are poorly studied. Studies to date have been focused either on particular parasite groups such as the Digenea (Madhavi, 1974a, 1974b, 1975, 1977 and Madhavi, 2011) or monogenea (Kritsky *et al*, 2012; Gudivada and Vankara, 2020) or cestoda (Subhapradha, 1948; Vijayalakshmi *et al*, 1995) or acanthocephalan (Gudivada *et al*, 2010), or more recently have examined the diversity of all helminth parasites found in or on speciûc families of ûsh such as the Scomberidae and Polynemidae (Madhavi and Rukmini, 1992; Madhavi and Sairam, 2000; Madhavi and Lakshmi, 2012; Gudivada *et al*, 2012, 2013; Gudivada and Vankara, 2010). The life-cycle patterns of the eucestodes are of prime importance as they have a considerable phylogenetic importance. But unfortunately the complete life-cycle patterns of both Trypanorhynchid and tetraphyllidean are not known yet. Conversely, the larval stages also have scolex features such as distinctive tentacular armature similar to orders of cestodes found in marine fish, which are indistinguishable to those found in the adult and enables specific morphological identification. The presence of a scolex bearing 2 or 4 bothria (Southwell, 1929 and Jones *et al*, 2004) and a tentacular apparatus with four retractile tentacles decorated with hooks as extensions of tentacle sheaths that are attached to four bulbs are the diagnostic features of the order Trypanorhyncha Diesing, 1863 (Palm, 2004 and Lee *et al*, 2016). The antagonistic bulbs and retractor muscles serve as holdfasts while the bothria are used for movement. Tetraphyllidean metacestodes from the

intestines of the teleost fish are complicated to identify due to their comparatively unremarkable scolex morphology, which lacks the diagnostic features of the adult cestodes. The genus Scolex Mueller, 1788 was proposed as a collective group to accommodate these metacestodes of unknown generic affinity (McDonald and Margolis, 1995) and the names *Scolex pleuronectis* Mueller, 1788 or *Scolex polymorphus* (Rudolphi, 1819) were used to describe them (Stunkard, 1977; Wardle and McLeod, 1952). The marine teleosts found in Visakhapatnam coast, Bay of Bengal are frequently infected with the larval stages (plerocerci, merocerci or plerocercoids of cestodes of the order Trypanorhyncha and *Scolex pleuronectis* of the order Tetraphyllidae, the adults of which are found in the stomach or spiral valves of elasmobranchs (Chervy, 2002). The presence of larval stages within the body cavity, musculature and gill arches of the fish can cause a great economic loss as their occurrence on fish gives an unpleasant appearance to the consumers (Palm, 2000; Beveridge *et al*, 2014; Abdelsalam *et al*, 2016). They signify an imperative constituent of parasite diversity but have repeatedly been overlooked due to taxonomic obscurity in classification (Palm, 2004). The taxonomic studies of this larval group of parasites has been carried out in only certain parts of the world such as Gulf of Mexico (Jensen, 2009), Persian Gulf (Haseli *et al*, 2011), Gulf of California, Java, Borneo, Australia and Hawaii (Carvajal *et al*, 1976; Palm, 2000; Jakob and Palm, 2006; Palm and Bray, 2014; Beveridge *et al*, 2014, 2017; Kipp *et al*, 2021), Madras and Visakhapatnam coast of bay of Bengal (Subhapradha, 1948; Anantharaman, 1963; Anantharaman and Krishnaswami, 1958; Chandra, 1985, 1986; Chandra and Rao, 1984; Vijayalakshmi, 1995) and Red sea (Nahed, 2005; Ali and Furhan, 2011; Mahmoud *et al*, 2015; Saleh *et al*, 2019). In the present study, larval cestodes of the order Trypanorhynchidae and Tetraphyllidae were described from the polynemid fishes of Visakhapatnam coast, Bay of Bengal.

METHODS

Study site

Six species of Marine threadfin fish belonging to four genera of the family Polynemidae (n= 1456) *i.e*., *Polydactylus sexfilis* (n = 115), *Polydactylus sextarius* (n = 676), *Polydactylus plebeius* (n = 77), *Filimanus heptadactyla* (n = 43), *Leptomelanosoma indicum* (n = 55) and *Eleutheronema tetradactylum* (n = 490) were collected from boat seine and trawlers of fishing harbour, fish landing centers and fish markets in and around Visakhapatnam coast (17.67°'N & 83.32°'E), Bay of Bengal between July 2005 and June 2007 for a period of two years (Fig. 1). The fishes were dead at the time of the collection. All the ûshes were measured, weighed and photographed for identification (Froese and Pauly, 2009).

Ethics and consent

The ethics and consent pertaining to the use of fish for this research were considered redundant according to the Animal ethics committee (CPCSEA) as the fish was dead at the time of collection, edible and was easily available to carry out research. Meanwhile the number of fishes used for this research was regulated by the Institute (Andhra University).

Sample collection and identification of the parasite

Morphometric and meristic data of the hosts were cautiously recorded on specifically prepared data sheets, along with their spectrum of larval parasites. Before dissecting the fish, its length, weight and sex were noted.

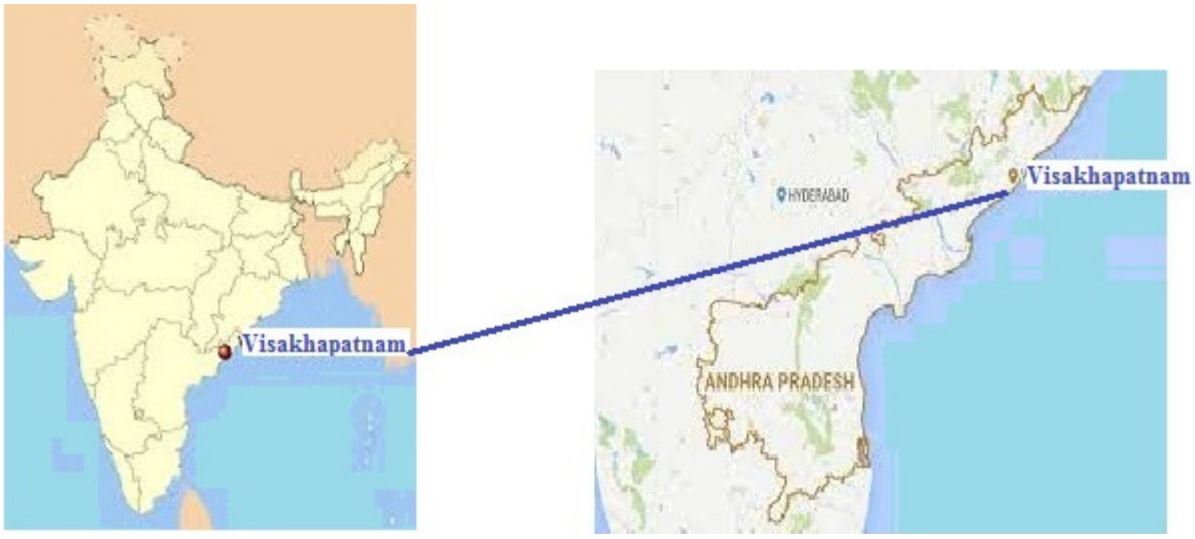

Fig. 1 : Map showing the study area- Visakhapatnam Coast, Andhra Pradesh.

Various internal organs such as stomach, intestine, muscles and heart were screened for larval tapeworms. The larval tapeworms were generally found attached to the stomach and intestinal walls of the host with the help of scolex in the present study. Intestine was detached cautiously and placed in a saline solution, then teased and contents were washed and observed under a stereozoom microscope. Standard protocols were followed to preserve the collected specimens in an ideal fixative, A.F.A (Alcohol-85%, Formalin- 5%, Glacial acetic acid- 10%) for 24 hours for the permanent slide preparation and further stained with alum carmine followed by dehydration with a graded series of alcohols (70%, 80%, 95%, 100%), xylene clearing and lastly mounted on a glass slide with Canada balsam or DPX mount. Parasites were observed, identified and captured in microphotographs taken with Nikon microscope at 10X, 30X and 40X magnifications and scale was given accordingly. Figures were drawn with the assistance of Reiss camera lucida (Figs.11-17) using 4X, 10X, 15X and 40X magnifications. All measurements were expressed in millimeters, (figs.2-10a) unless otherwise mentioned.

RESULTS

Scolex pleuronectis stages of the Order Tetraphyllidae

A total 3870 larval *Scolex pleuronectis* stages of the order Tetraphyllidae could be recovered from the stomach and intestine of *P. sextarius* (n = 3511), *P. plebeius* (n = 62), *P. sexfilis* (n = 37), *Eleutheronema tetradactylum* (n = 228) and *Leptomelanosoma indicum* (n = 32), but there were no reports of larval cestodes from *Filimanus heptadactyla* during the present study.

***Scolex pleuronectis*- I Mueller, 1787** (Based on 20 specimens) **(Fig. 2,11)**

The larva is usually short, thick and flattened. It measures 1.008-1.280 × 0.400. Pars bothridialis is short measuring 0.272 in length. There are four oval bothridia on the scolex, each measuring 0.144 × 0.112 in width. Apical sucker is large, slightly depressed, and cup shaped. It measures 0.080 in diameter. Body is translucent and contains refringent calcareous granules of different sizes. Osmoregulatory canals can be observed clearly running posteriorly, through the sides.

***Scolex pleuronectis*- II** (Based on 20 specimens) **(Fig. 3,12)**

The plerocercoid is short, thick, flattened and blunt posteriorly. It measures 0.88-0.1 × 0.34-0.41. Pars bothridialis is short measuring 0.23-0.25 in length. There are four oval bothridia on the scolex, each measuring 0.06 in diameter. Apical sucker is large, round and slightly projected. It measures 0.08 in diameter. Body is translucent and large number of refringent calcareous granules could be observed within it. Osmoregulatory system could be seen clearly.

***Scolex pleuronectis*- III** (Based on 20 specimens) **(Fig. 4)**

Worms are cylindrical with slight constriction behind the bothridia. The body measures 0.09-0.1 × 0.34-0.41. Scolex with four oval bothridia, each measuring 0.09 in diameter. Pars bothridialis measures 0.20-0.23 in length. Apical sucker muscular, large and round. It measures 0.08 in diameter. Body is filled with refringent calcareous granules. Excretory canals are conspicuous.

***Scolex pleuronectis*-IV** (Based on 20 specimens) **(Fig.5, 13)**

The larva is provided with an evaginated scolex with a rostellum like protruberance. Elongated body measures 1.008-1.28 × 0.4. Pars bothridialis measures 0.272 in length. Apical sucker is 0.080 in diameter. Bothridia are sessile, oval and each measures 0.144 × 0.112. The translucent body is filled with refringent calcareous granules. Excretory canals are conspicuous.

***Scolex pleuronectis* – V** (Based on 20 specimens) **(Fig. 6)**

These larvae are elongate, fusiform, with segmented body. Scolex evaginated with a rostellum like protruberance. Larvae measure 0.23-0.25 × 0.03-0.04. Pars bothridialis measures 0.07-0.08 in length. Bothridia are sessile and oval in shape. They measure 0.02-0.03 × 0.01-0.02. The anterior protruberance measures 0.02-0.03. There are three segments in the body and the posterior segment is larger. The first segment measures 0.04, second segment 0.02, and the posterior one is 0.06 in length. Body is filled with refringent calcareous granules. Excretory canals are conspicuous.

***Scolex pleuronectis bilocularis* Wagener, 1854** (Based on 20 specimens) **(Fig. 7, 14)**

These are elongate and taper posteriorly. They measure 2.160 × 0.112. Pars bothridialis is 0.240 in length. There are four biloculate bothridia. In each bothridium, the first loculus measures 0.064 × 0.032 and the second 0.048 × 0.048. Apical sucker is muscular and 0.096 in diameter. Body is translucent and is with refringent calcareous granules of different sizes. Excretory canals are conspicuous running posteriorly along the sides.

***Scolex pleuronectis trilocularis* Wagener, 1854** (Based on 20 specimens) **(Fig. 8,15)**

This larva has somewhat elongated and cylindrical

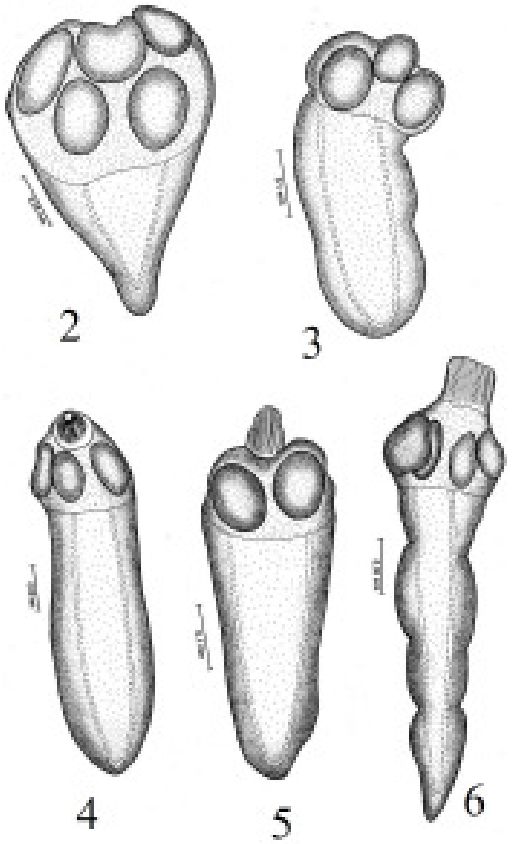

Fig. 2 : *Scolex pleuronectis*- I, 400X. **Fig. 3 :** *Scolex pleuronectis*- II, 200X. **Fig. 4 :** *Scolex pleuronectis*- III, 400X. **Fig. 5 :** *Scolex pleuronectis*- IV, 200X. **Fig. 6 :** *Scolex pleuronectis*- V, 400X.

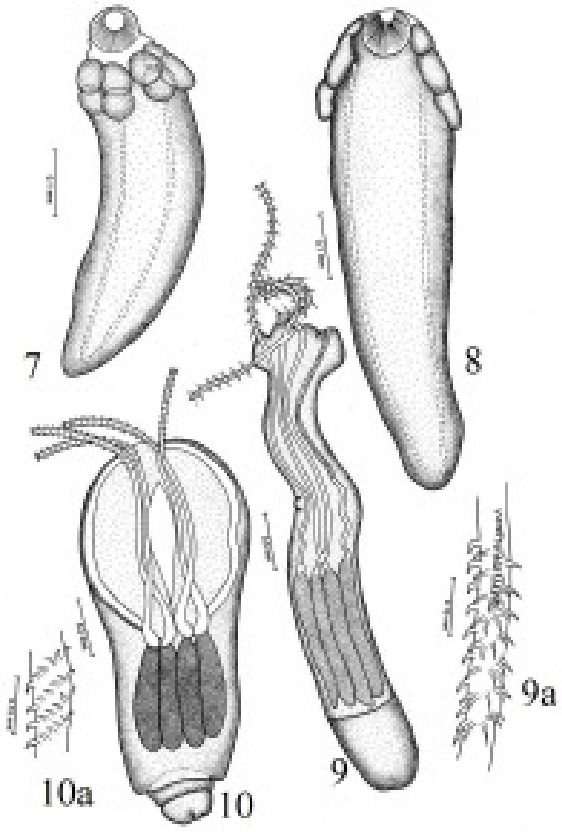

Fig. 7 : *Scolex pleuronectis bilocularis*, 200X. **Fig. 8 :** *Scolex pleuronectis trilocularis*, 200X. **Fig. 9 :** Trypanorhynchid sp. larva, 40X. **Fig. 9a :** Arrangement of hooks, 150X. **Fig. 10 :** *Nybelinia* sp. larva, 100X. **Fig. 10a :** Arrangement of hooks, 150X.

body. Pars bothridialis measures 0.176 in length. Total body measures 1.760 × 0.192. Bothridia are triloculate. Upper loculus measures 0.048 × 0.048, middle loculus measures 0.064 × 0.080 and the lower or posterior measures 0.064 × 0.064. The muscular apical sucker is 0.048 in diameter. Body is transparent and filled with refringent calcareous granules.

Plerocercoid of *Trypanorhynchid* spp. (Based on 10 specimens) **(Fig.9, 9a,16)**

15 larvae were collected from *P. sexfilis* (n= 6), *P. sextarius* (n= 4) and *F. heptadactyla* (n = 5). These larvae found attached to the intestinal wall as cysts. Larvae long, thick and cylindrical in shape, measuring 3.814-3.878 × 0.552-0.579. Body consists of anterior scolex and posterior cyst. Scolex consists anteriorly bothridial region with diffuse bothridia. Pars bothridialis measures 0.421-0.473. Pars vaginalis is long and tubular, measuring 1.683-1.736. It bears two pairs of long proboscid sheaths. Hooks are dense in the anterior part of proboscid sheaths. These regions are armed with oblique rows of strongly curved metabasal hooks, measuring 0.03-0.05, arranged 16 hooks in each oblique row, chainnette of hooks present on external face. Pars bulbosa is shorter than the pars vaginalis and wide, measuring 1.420-1.473. It consists of four, oval, diffuse proboscid sacs. Each proboscid sac measures 1.298-1.341. Cyst is thin, broad, transparent and white in colour.

Larva of *Nybelinia* Poche, 1926 (Based on 10 specimens) **(Fig. 10, 10a,17)**

17 larvae were found encysted in the walls of the stomach of *P. sexfilis* (n = 8) and *F. heptadactyla* (n = 9). Cysts are round, oval and milky white in colour. The worms were released from the cysts for the preparation of permanent slides. Worms are pear shaped measuring 0.97-0.99x0.6-0.7. Greatest width is at the level of bothridia which are sessile with thickened edges and four in number. Pars bothridialis measures 0.25-0.27 × 0.33-0.35. Bothridia compress into the head measuring 0.25-0.26 × 0.06-0.08. Pars vaginalis shorter than pars bothridialis measuring 0.20-0.22. Pars vaginalis possesses tentacular sheaths, which are long, slender and not sinuous. Basal part of tentacular sheaths bears prebulbular organs. Pars bulbosa is shorter than pars vaginalis measuring 0.30-0.33 in length. It consists of bulbs which are banana shaped and lie slightly away from the base of the scolex. Each bulb measures 0.24-0.26 × 0.04-0.06.

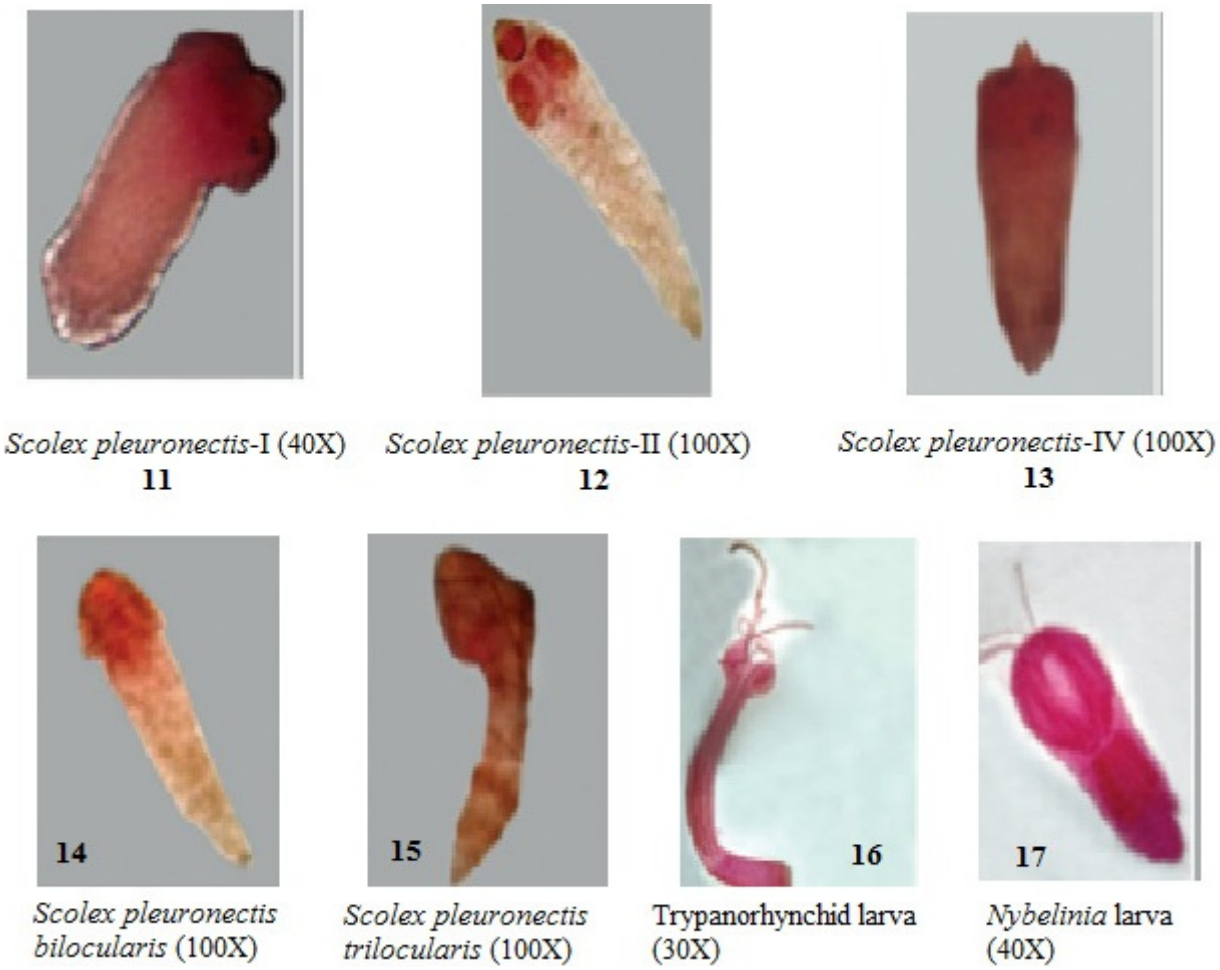

Fig. 11 : Microphotograph of *Scolex pleuronectis*- I, 40X. **Fig. 12 :** Microphotograph of *Scolex pleuronectis*- II, 100X. **Fig. 13 :** Microphotograph of *Scolex pleuronectis*- IV, 100X. **Fig. 14 :** Microphotograph of *Scolex pleuronectis bilocularis*, 100X. **Fig. 15 :** Microphotograph of *Scolex pleuronectis trilocularis*, 100X. **Fig. 16 :** Microphotograph Trypanorhynchid sp. larva, 30X. **Fig. 17 :** Microphotograph of *Nybelinia* sp. larva, 40X.

Pars post bulbosa measures 0.08-0.10 in length. Four tentacles emerge out from the bothridia and measure 0.18-0.20 × 0.01-0.02. Tentacles armed with simple, delicate, very minute, curved and spirally planned hooks. Each row consists of 12 hooks. They are all uniform in size. There is a peculiar fold of collar which hangs back from the head and across the anterior part of the appendix.

DISCUSSION

The study of systematics of larval cestodes of fishes and birds was started way back from 16th century (Redi, 1684; Bloch, 1779; Fabricius, 1780 and Van Beneden, 1850). Wardle and McLeod (1952) also described the classification, structure and biology of cestodes of the world with their identification key in detail. But, the presence of larval cestodes in marine fishes was first noticed by Mueller (1787), who named them as Scolex as their scolex was not well differentiated to identify them to species level. Later Stiles and Hassal (1912) named these larvae as *Scolex pleuronectis* Mueller, 1787 which was accepted by all scientists and such larval stages later discovered were named as *Scolex pleuronectis*. Dollfus (1923, 1924, 1931, 1942, 1974), Yamaguti (1934) and Stunkard (1977) reported a number of such different larvae from different hosts. However, Rudolphi (1819) doubted on the validity of *Scolex pleuronectis* and chose *S. polymorphus* to synonymize these species. Scientists have gradually realized that these two names actually include a large number of tetraphyllidean metacestodes (Chambers *et al*, 2000). In India, Anantaraman and Krishnaswami (1958), Anantaraman (1963), Rao and Madhavi (1966), Chandra and Rao (1984), Radha Krishnan and Nair (1984), Vijaya lakshmi *et al* (1995) and Silva *et al* (2000) reported *Scolex pleuronectis* from different invertebrates and vertebrates. In the present study, seven different stages of *Scolex pleuronectis* were collected from *P. sextarius*, *E. tetradactylum*, *P. sexfilis* and *P. plebeius*. These were numbered as *Scolex pleuronectis* I to V and one larva as *Scolex pleuronectis bilocularis* and another

as *Scolex pleuronectis* trilocularis. These larvae occurred quite frequently in these fishes and in large numbers. The presence of these larvae in large numbers suggests the harm done to the host. Similarly, the plerocercoids of Tetraphyllidea and Trypanorhyncha from fishes of India were described by Southwell (1930) and their occurrence in intermediate hosts (copepods, marine fish, ctenophores, molluscs and others) were described by Hyman (1951), Yamaguti (1959) and Anantaraman (1963). The occurrence of tetraphyllid larva in copepods of Bay of Bengal was recorded by Anantaraman and Krishnaswami (1958) and Rao and Madhavi (1966) while the occurrence of trypanorhynchid plerocercoids of *Callitetrarhynchus gracilis*, *Gilquinia* spp, *Gymnorhynchus* spp. and *Lacistorhynchus* spp. from teleost fishes of Bay of Bengal was described by Chandra (1985). The present parasites were collected from *P. sexfilis* and *F. heptadactyla* from this coast. Present parasites could not be identified up to the genus or species level as the arrangement of hooks is not clear. So, they are named as Pleroceroid of Trypanorhynchid larva. The occurrence of trypanorhynchid larval forms in teleost fish is not new. Trypanorhynchid larval stages occur usually in encysted stages. Cysts are transparent and elastic can be mounted either together with cyst wall or breaking the cyst. Unlike tetraphyllid and other larval forms, trypanorhynchid larvae are with very well developed scolex which can be identified upto genus level and sometimes even up to species level. The third larval species belonged to the genus *Nybelinia*, which was first erected by Poche (1926). In the present study these larval forms conform to *Nybelinia* in all characters though identification up to species level is not possible. Cestode larval forms were mostly reported from invertebrates. There were reports of *Nybelinia* from teleosts by Carvajal *et al* (1976), Shah and Bilquees (1979), Chandra (1986) and Vijayalakshmi *et al* (1995). In the present study, these larval forms are identified as the larval stage of *Nybelinia.* The presence of these cestode larvae in a fish suggests the role of these fish as an intermediate host for a definitive host in which these larvae metamorphose to adult stages. Since adult tetraphyllideans and trypanorhynchids parasitize only elasmobranch fish, the occurrence of these larvae suggests that these fish are preyed by elasmobranch fish.

CONCLUSION

The present study proclaims that the polynemid fishes of Visakhapatnam coast, Bay of Bengal has a wide range of undisclosed larval tapeworm biodiversity, which is yet to be unveiled. The identification of many larval cestodes for the first time from marine threadfin fish paves a pathway to conduct many more parasitological surveys to unveil the parasite diversity of this geographical location and will be immeasurably useful to construct a catalog on host-parasite association in the Bay of Bengal.

Declarations

Ethics approval and consent to participate.

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interest

The authors declare that they have no competing interest related to the work.

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Author's contribution

The first author MG was involved in collecting the fish samples and parasites, literature collection and the second and third authors PNY and APV helped in writing and drafting the manuscript. All authors have read and approved the manuscript.

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List of all abbreviations

ZDAU- Museum, Zoology Department, Andhra University
CPCSEA- Control and Supervision of Experiments on Animals
UGC, SERO- University Grants Commission, South East Regional Office
FDP- Faculty Development Programme